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An Investigation of Larval Ascaridoid Nematodes in Some Marine Fish from the Gulf of Thailand

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ABSTRACT

Larval Ascaridoid nematodes were investigated from in 1,880 samples from 78 species of marine fish from the Gulf of Thailand. Four genera of larval Ascaridoid nematodes: *Anisakis, Contracaecum, Porrocaecum* and *Rhaphidascaris* were found in the mesenteries, visceral organs and the intestines of 57 samples of 11 marine fish species. Larvae of the genus *Anisakis* were found in four species of fish (*Muraenesox* sp., *Epinephelus areolatus, Rachycentron canadum*, *Trichiurus lepterus*). Larvae of the genus *Contracaecum* were found in two species of fish (*Priacanthus tayenus*, *Scolopsis dubiosus*). Larvae of the genus *Porrocaecum* were found in two species of fish (*Priacanthus tayenus*, *Seriolina nigrofasciata*). Larvae of the genus *Rhaphidascaris* were found in four species of fish (*Dasyatis* sp., *Plotosus anguillaris*, *Johnius dussumieri, Pelates quadrilineatus*). *Priacanthus tayenus* was infected with the highest number of species of Ascaridoid nematodes (2 genera). The highest number of Ascaridoid nematodes per fish was observed in *Muraenesox* sp., which was infested with 3.75 *Anisakis* per fish. In this study, *Anisakis* followed by *Rhaphidascaris*, were found in 63 (52.07%) and 32 (26.45%) fish samples, respectively.

Key words: parasite, nematode, Ascaridoid, the gulf of Thailand

INTRODUCTION

Nematodes are invertebrate roundworms that inhabit moist interstitial environments in all habitats from the sea, to freshwater and land, including the bodies of plants and animals (Brusca and Brusca, 1990; Ruppert *et al*., 2004). They comprise 256 families and more than 40,000 species (Nagasawa, 2005). There are many parasitic nematodes displaying all degrees of parasitism and attacking virtually all plants and animals, often in species-specific relationships. The numerous species that infest humans, food and horticultural crops and domesticated animals make nematodes one of the most important parasitic animal taxa (Ruppert *et al*., 2004). Nematodes of the Ascaridoidea naturally parasitize fish, cephalopods, marine mammals and piscivorous birds. Humans can also become accidental hosts by ingesting raw marine fish and invertebrates infested with third stage larvae (Anderson, 1992; Szostakowksa *et al*., 2002; Doupé *et al*., 2003). In humans, the ingestion of third-stage larvae of some species of the Ascaridoidea can cause the disease known as anisakiasis. The nematodes that cause anisakiasis are larvae of *Anisakis simplex* (frequently called *Anisakis* type I larvae) in most cases, followed by larvae of *Pseudoterranova*

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decipiens. Other larval anisakid nematodes, such as *A. physeteris* (*Anisakis* type II larvae), *Contracaecum* spp., *Contracaecum osculatum* and *Heterothylacium aduncum*, are very rarely found in humans (Schaum and Müller, 1967; Ishikura, 2003).

Anisakis, Pseudoterranova and *Contracaecum* have similar life cycles (Lymbery *et al*., 2002). Marine mammals and piscivorous birds are definitive hosts, while aquatic invertebrates and fish are intermediate hosts. In the intermediate host, the larvae penetrate the intestine and invade the celomatic cavity or the muscle, where they molt into the third stage and become encapsulated (Bernardi, 2009). Based on the definitive hosts, in which adult worms occur, *A. simplex* and *P. decipiens* are called whaleworm and sealworm, respectively (Nagasawa, 2005).

Since the first discovery in 1955, of humans infected by *Anisakis* larvae in the Netherlands, more than 13,000 cases of the disease caused by anisakid worms have been reported from nearly 20 countries up until early 1990 (Ishikura *et al*., 1992). The areas of highest prevalence are Scandinavia (from cod livers), Japan (after eating sushi and sashimi), the Netherlands (by eating infected fermented herrings (Maatjes) and along the Pacific coast of South America (from eating ceviche) (Wikipedia, 2008). About 20,000 cases of anisakiasis were reported in the literature, with over 90% from Japan and the rest from the European Union, United States of America, Canada, New Zealand, Chile and Egypt (Chai *et al*., 2005; Bernardi, 2009). Anisakiasis occurs when humans ingest raw or lightly cooked marine fish and invertebrates that are infested with anisakis larvae (Nagasawa, 2005). The larvae invade the gastrointestinal mucosa and cause abdominal pain, nausea, vomiting and various gastrointestinal lesions (Kim *et al*., 2006). The abdominal pain usually occurs 2 to 7 h after eating raw seafood containing *Anisakis* larva. The severity of the pain is different among patients (Nagasawa, 2005). Although, this group of nematodes is harmful to humans, scientists use some species of Ascaridoid nematodes (*Anisakis)* as a biological tag for host-stock characterization (Mattiucci *et al*., 2008).

In this research, 78 marine fish species from the Gulf of Thailand were observed for Ascaridoid nematodes. The results obtained from this research can be used as basic data for anisakiasis prevention in Thailand.

MATERIALS AND METHODS

Seventy-eight marine fish species (1,880 samples) were collected between December 2006 and December 2007 from the Gulf of Thailand. Samples were collected from commercial catches and individual fishermen in Chon Buri province. Identification of the fish was undertaken according to Collette and Nauen (1983), Russell (1990), McKay (1992), Nakamura and Parin (1993), Rainboth (1996) and Nelson (2006). The internal organs of the fish were removed and examined for nematode parasites under a stereo microscope. Parasites were removed from the hosts using fine point forceps, preserved in 70% ethyl alcohol and cleared in glycerine for further examination. Samples were studied using light microscopy, manipulation and measuring techniques.

Ascarididans (Order Ascaridida) are characterized by the presence of three prominent lips, external labial papillae and numerous caudal papillae. Identification of Ascaridoid nematodes was based on morphological features: the presence of ventriculus, ventricular appendix, intestinal cecum, the length of the intestinal cecum, the number and arrangement of the caudal papillae, the size of the spicules and the morphology of the tail tip according to Bykhovskaya–Pavlovskaya *et al*. (1964), Hoffman (1967), Moravec (1994). Prevalence and mean intensity of each parasitic species were determined as described in Margolis *et al*. (1982).

RESULTS AND DISCUSSION

A total of 1,880 fish samples from 78 marine fish species were collected from the Gulf of Thailand. Eleven species of fish were infested with larval Ascaridoid nematodes. There were four genera of larval Ascaridoid nematodes: *Anisakis, Contracaecum, Porrocaecum* and *Rhaphidascaris* found in the mesenteries, visceral organs and the intestines.

The Ascaridoid nematodes were classified into the order Ascaridida, superfamily Ascaridoidea, family Anisakidae (*Anisakis*, *Contracaecum*, *Raphidascaris*) and the family Ascarididae (*Porrocaecum*) (De Ley *et al*., 2006). Larvae of the genus *Anisakis* were found in four species of fish (*Muraenesox* sp., *Epinephelus areolatus, Rachycentron canadum*, *Trichiurus lepterus*). Larvae of the genus *Contracaecum* were found in two species of fish (*Priacanthus tayenus*, *Scolopsis dubiosus*). Larvae of the genus *Porrocaecum* were found in two species of fish (*Priacanthus tayenus*, *Seriolina nigrofasciata*). Larvae of the genus *Rhaphidascaris* were found in four species of fish (*Dasyatis* sp., *Plotosus anguillaris*, *Johnius dussumieri, Pelates quadrilineatus*). The highest diversity of parasites was found in *Priacanthus tayenus* with 2 genera of Ascaridoid nematodes, *Contracaecum* and *Porrocaecum*. The highest number of Ascaridoid nematode parasites occurred in *Plotosus anguillaris* followed by *Trichiurus lepterus* and *Priacanthus tayenus* with 47.50, 30.00 and 23.53% infection, respectively (Figure 1). The mean intensity of *Anisakis* sp. in *Muraenesox* sp. was the greatest, followed by *Anisakis* sp. in *Trichiurus lepterus* and *Porrocaecum* sp. in *Priacanthus tayenus*, with 3.75, 3.00 and 2.25, respectively (Table 1).

In this study, *Anisakis* was found in the highest number of fish samples (63; 52.07%) followed by *Raphidascaris* (32; 26.45%) and *Contracaecum* (16; 13.22%) (Figure 2).

All of the Ascaridoid nematodes were found in visceral organs, including the peritoneum. None of the parasites were found in the flesh of fish, as this group of parasites prefers to inhabit visceral organs (Lymbery *et al*., 2002).

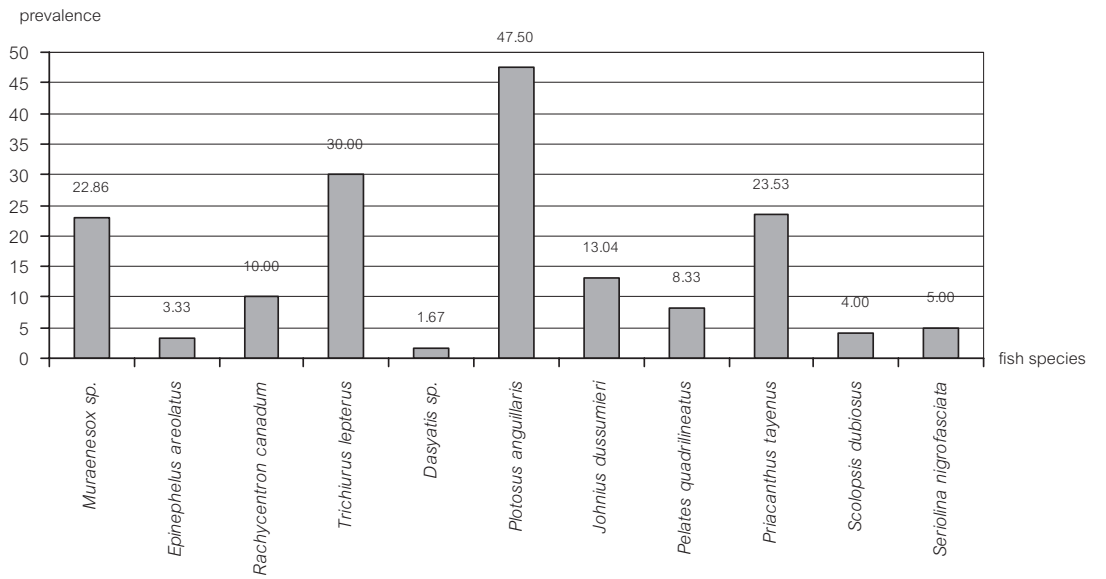

Figure 1 Prevalence of Ascaridoid nematodes in some marine fish species from the Gulf of Thailand.

Table 1 Prevalence and mean intensity of Ascaridoid nematodes in some marine fish from the Gulf of Thailand.

Fish species	No. of fish	Parasite genera	Family	No. of infected fish	Prevalence	Mean intensity
	9	-	-	-	-	-
Arius sp.	20	-	-	-	-	-
Batrachus grunniens	2	-	-	-	-	-
Caranx armatus	1	-	-	-	-	-
Caranx hippos	45	-	-	-	-	-
Caranx malam	45	-	-	-	-	-
Chiloscyllium punctatum	60	-	-	-	-	-
Chorinemus lysan	6	-	-	-	-	-
Cynoglossus bilineatus	30	-	-	-	-	-
Dasyatis sp.	60	*Raphidascaris* sp.	Anisakidae	1	1.67	1.00
Drepane punctata	45	-	-	-	-	-
Dussumieria hasseltii	45	-	-	-	-	-
Eleutheronema tetradactylum	10	-	-	-	-	-
Epinephelus areolatus	30	*Anisakis* sp.	Anisakidae	1	3.33	1.00
Epinephelus faveatus	5	-	-	-	-	-
Epinephelus tauvina	25	-	-	-	-	-
Euthynnus affinis	8	-	-	-	-	-
Gaterin diagrammus	2	-	-	-	-	-
Gazza minuta	25	-	-	-	-	-
Gerres filamentosus	19	-	-	-	-	-
Gerres oyena	20	-	-	-	-	-
Hilsa kanagurta	20	-	-	-	-	-
Holocentrus rubrum	5	-	-	-	-	-
Hyporhamphus gaimardi	40	-	-	-	-	-
Johnius dussumieri	23	*Raphidascaris* sp.	Anisakidae	3	13.04	1.33
Johnius soldado	20	-	-	-	-	-
Lagocephalus spadiceus	1	-	-	-	-	-
Lates calcarifer	40	-	-	-	-	-
Leiognathus sp.	60	-	-	-	-	-
Liza dussumieri	8	-	-	-	-	-
Liza vaigiensis	4	-	-	-	-	-
Lutianus johni	2	-	-	-	-	-
Lutianus vitta	20	-	-	-	-	-
Monacanthus chinensis	40	-	-	-	-	-
Muraenesox sp.	60	*Anisakis* sp.	Anisakidae	14	22.86	3.75
Nemipterus furcosus	14	-	-	-	-	-
Nemipterus hexodon	250	-	-	-	-	-
Ophichthys sp.	12	-	-	-	-	-
Otolithes rubber	20	-	-	-	-	-
Pampus argenteus	15	-	-	-	-	-
Parachaetodon ocellatus	2	-	-	-	-	-
Parastromateus niger	15	-	-	-	-	-

Table 1 Prevalence and mean intensity of Ascaridoid nematodes in some marine fish from the Gulf of Thailand (continued).

Fish species	No. of fish	Parasite genera	Family	No. of infected fish	Prevalence	Mean intensity
Parupeneus sp.	6	-	-	-	-	-
Pelates quadrilineatus	15	*Raphidascaris* sp.	Anisakidae	1	8.33	2.00
Platax orbiculasis	1	-	-	-	-	-
Platax teira	1	-	-	-	-	-
Platycephalus indicus	30	-	-	-	-	-
Plectorhynchus pictus	4	-	-	-	-	-
Plotosus anguillaris	40	*Raphidascaris* sp.	Anisakidae	19	47.50	1.26
Plotosus canius	16	-	-	-	-	-
Pomadasys maculatus	2	-	-	-	-	-
Priacanthus tayenus	34	*Contracaecum* sp.	Anisakidae	8	23.53	1.88
		Porrocaecum sp.	Ascarididae	4	11.76	2.25
Psammoperca waigiensis	1	-	-	-	-	-
Psettodes erumei	34	-	-	-	-	-
Rachycentron canadum	10	*Anisakis* sp.	Anisakidae	1	10.00	1.00
Rastrelliger kanagurta	30	-	-	-	-	-
Rastrelliger neglectus	20	-	-	-	-	-
Saurida micropectoralis	60	-	-	-	-	-
Scatophagus argus	70	-	-	-	-	-
Scolopsis dubiosus	25	*Contracaecum* sp.	Anisakidae	1	4.00	1.00
Scomberomorus guttatus	15	-	-	-	-	-
Selaroides leptolepis	15	-	-	-	-	-
Seriolina nigrofasciata	20	*Porrocaecum* sp.	Ascarididae	1	5.00	1.00
Siganus canaliculatus	25	-	-	-	-	-
Siganus javus	25	-	-	-	-	-
Sillago maculate	20	-	-	-	-	-
Sillago sihama	30	-	-	-	-	-
Sparus berda	1	-	-	-	-	-
Sphyraena obstusata	12	-	-	-	-	-
Stolephorus sp.	2	-	-	-	-	-
Synaptura quagga	3	-	-	-	-	-
Therapon jarbua	65	-	-	-	-	-
Therapon theraps	20	-	-	-	-	-
Trichiurus lepterus	10	*Anisakis* sp.	Anisakidae	3	30.00	3.00
Trypauchen vagina	20	-	-	-	-	-
Tylosurus annulata	2	-	-	-	-	-
Valamugil buchanani	12	-	-	-	-	-
Unidentified sp.1	1	-	-	-	-	-
Total	**1,880**					

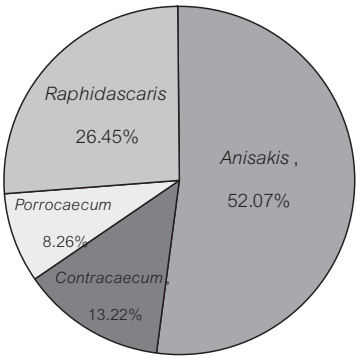

Figure 2 Percentage of larval Ascaridoid nematodes in each genera found in this study.

In Thailand, many species of larval Ascaridoid nematodes such as *Anisakis*, *Contracaecum* and *Raphidascaris* have been found in: *Apogon* sp., *Caranx crumenophthalmus*, *Caranx gymnostethoides*, *Caranx leptolepis*, *Caranx malabaricus*, *Decapterus russelli*, *Dussumieria hasseltii*, *Euthyannus affinis*, *Leiognathus elongates*, *Lutianus lineolatus*, *Magalaspis cordyla*, *Nemipterus delagoae*, *Nemipterus hexodon*, *Nemipterus japonicus*, *Priacanthus tayenus*, *Pseudupeneus luteus*, *Rastrelliger kanagurta*, *Rastrelliger neglectus*, *Sardinella jussieu*, *Saurida tumbil*, *Scomberomorus commersoni*, *Sphyraena jello*, *Trichiurus haumela* and *Upeneus tragula* (Cheeramakara, 1977; Bhaibulaya, 1981; Leethochavalit, 1987). As found in this study, *Priacanthus tayenus* is often infested with Ascaridoid nematodes.

Although, some species of Ascaridoid nematode do not have any record of causing disease in humans, the presence of *Anisakis* larvae on and in the viscera and flesh may impact upon visual aesthetics and the market value, and parasite removal only adds to product cost while further reducing its attraction to consumers (Doupe′ *et al.*, 2003). Moreover, in some genera, such as *Porrocaecum*, *Raphidascaris* can cause disease in other animals such as fish and birds (Lymbery *et al.*, 2002). Hence, there is the a risk in using fish that are infested with larval Ascaridoid nematodes for animal feed.

To prevent human infection by *Anisakis* larvae, marine fish must be kept frozen under -20°C for at least seven days or cooked for a period long enough to raise the temperature of the innermost part of the flesh to 63°C for at least 15 sec (Adams *et al.*, 1997; Mercado *et al.*, 2001). The maximum survival period of *Anisakis* larva in 10% NaCl, dry salt, 35% ethyl alcohol, vinegar and lemon juice was 3 d, 1 h 20 min, 15 h, 8 d and 4 d, respectively (Cheeramakara, 1977).

CONCLUSION

Seventy-eight species (1,880 samples) of marine fish from the Gulf of Thailand were investigated. The results showed that 11 species in 57 fish samples (14.10%) were infested with larval Ascaridoid nematodes. Four genera of nematode parasites: *Anisakis, Contracaecum, Porrocaecum* and *Rhaphidascaris* were found in this study. *Anisakis* and *Contracaecum* are possible causes of disease in humans. Thus, sanitary regulations covering raw fish food products for human consumption are important to prevent anisakiasis in humans.

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